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REVIEW ARTICLE

Common Gene Rearrangements in Prostate Cancer

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ABSTRACT

Prostate cancer is a common heterogeneous disease, and most patients diagnosed in the post prostate-specific antigen (PSA) era present with clinically localized disease, the majority of which do well regardless of treatment regimen undertaken. Overall, those with advanced prostate cancer at time of diagnosis do poorly after androgen withdrawal therapy. Understanding the biologic underpinning of prostate cancer is necessary to best determine the risk of disease progression and would be advantageous for the development of novel therapeutic approaches to impede or prevent disease. This review focuses on the recently identified common *ETS* and non-*ETS* gene rearrangements in prostate cancer. Although multiple molecular alterations have been detected in prostate cancer, a detailed understanding of gene fusion prostate cancer should help explain the clinical and biologic diversity, providing a rationale for a molecular subclassification of the disease.

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INTRODUCTION

Prostate cancer is a major public health problem in the United States with more than 217,000 cases diagnosed and more than 32,000 deaths in 2010.[1] Currently, a high percentage of men diagnosed through prostate-specific antigen (PSA) testing will die with prostate cancer and not from it. The aging population, with an expected increase to more than 500,000 diagnosed prostate cancers per year by 2015, presents a key clinical problem: the determination of risk factors in the development of aggressive prostate cancer and avoidance of unnecessary overtreatment. Although effective surgical and radiation treatments exist for clinically localized disease, metastatic prostate cancer remains essentially incurable, and most men diagnosed with metastatic disease will succumb over a period of months to years.

One of the challenges in understanding prostate cancer has been the clinical and molecular heterogeneity associated with this common disease. Hematologic malignancies, such as acute myeloid leukemia, are often subtyped on the basis of the recurrent cytogenetic or molecular aberration identified. Therefore, the recent and surprising discovery that at least 50% of prostate cancers harbor recurrent gene rearrangements resulting in the fusion of genes[2] may enable molecular subtyping of prostate cancers, similar to what has been established for leukemias and lymphomas, thereby enabling the identification of patients with aggressive disease. Most often, these fusions juxtapose a hormone-specific promoter that acts as an "on" switch for the oncogene, conferring a distinct biology to this tumor. Although other molecular events play a role in prostate cancer development and progression, defining prostate cancer on the basis of the presence or absence of the different on switch that drives cancer development provides novel insight into disease heterogeneity. Despite the current lack of specific therapies to target the on switches created by the rearrangements, we contend that this hormonally controlled, clonal oncogenic event modulates tumor cells in a manner distinct from rearrangement-negative cases. The focus of this review is to determine the role of gene fusion in prostate cancer heterogeneity and provide a strong rationale for a molecular subclassification of this common tumor.

GENE FUSION PROSTATE CANCER: A PARADIGM SHIFT

Recurrent chromosomal aberrations were thought to be primarily characteristic of leukemias, lymphomas, and sarcomas. Epithelial tumors (ie, carcinomas), which are the most common human tumors contributing to a large percentage of morbidity and mortality associated with human cancer, comprised less than 1% of the known, disease-specific chromosomal rearrangements. Thus, the discovery of the *ETS* family transcription factor gene fusions by Tomlins et al[2] in 2005 dramatically changed the field of solid tumor biology. The recurrent *TMPRSS2-ERG* fusion in prostate cancer is now the most common rearrangement described in any neoplasm, considering the large number of cases diagnosed in

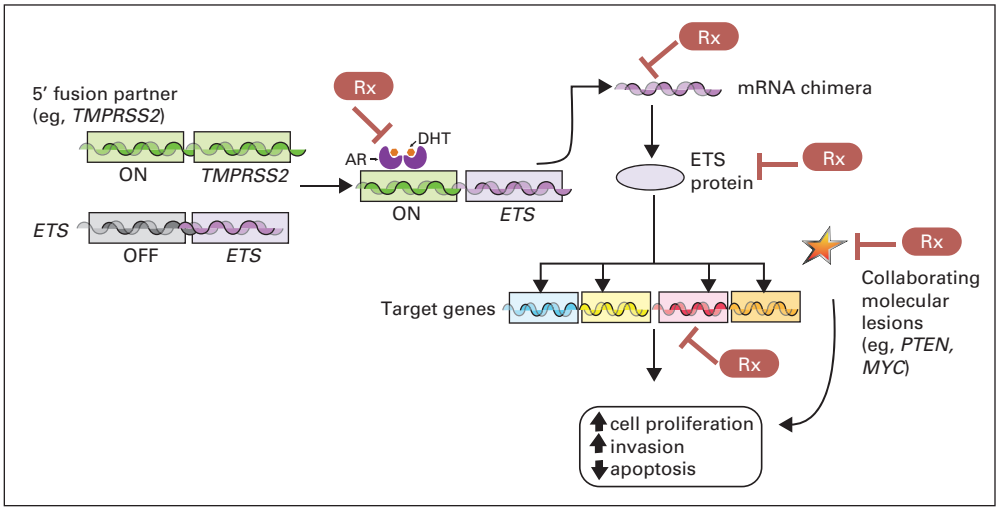

Fig 1. Targeting prostate cancer by using the gene fusion "on/off" switches. Gene fusion prostate cancers present an opportunity to target specific promoters and *ETS* genes. Several approaches can be considered in targeting gene fusion prostate cancers. In this schematic, the *TMPRSS2* 5′ promoter acts as an on switch in the presence of androgens and, in some settings, estrogen. Therefore, targeting the androgen receptor (AR) site of *TMPRSS2* with small molecules may effectively decrease the expression of the fusion transcript. Approaches to target the mRNA fusion transcript by using short interfering RNA (siRNA) might be an effective means of decreasing the specific oncogenic fusion transcript. Small molecules might also be generated against the specific 3′ *ETS* gene or putative collaborating lesions such as *PTEN* or *MYC*). DHT, dehydrotestosterone; Rx, drug therapy (eg, small molecules, siRNA).

the world each year. The greatest surprise to the research community was that such a common rearrangement would be found in the most prevalent non–skin cancer to afflict men.

TMPRSS2-ETS *Family Fusion Genes and Prostate Cancer*

The key to the discovery of *TMPRSS2-ETS* gene fusions was the development of a simple, statistical approach termed "cancer outlier profile analysis" (COPA) to identify oncogene profiles in a subset of samples within publicly available cancer profiling data sets, characteristic of genes commonly associated with known genomic rearrangements (reviewed by Rubin and Chinnaiyan[3] and Hanauer et al[4]). The application of COPA in prostate cancer microarray experiments revealed two consistently high-scoring and mutually exclusive candidates across 50% to 70% of prostate cancer samples that were members of the *ETS* family of transcription factors, *ERG* and *ETV1*. Further experiments revealing fusions of the 5′-untranslated region of *TMPRSS2* (21q22.3) with the *ETS* transcription factor family members—*ERG* (21q22.2), *ETV1* (7p21.2),[2] or *ETV4*[5]—were identified, suggesting a novel mechanism for overexpression of the *ETS* genes in prostate cancer. The discovery of a known family of oncogenic transcription factors driven by a hormonally regulated promoter offers critical therapeutic opportunities to target the on-off switches created by the rearrangement (Fig 1) and suggests that additional common epithelial cancers may harbor similar organ-specific rearrangements.

ETS *Gene Fusions in Prostate Cancer Progression*

Prostate cancer, like other cancers, develops in the background of diverse genetic and environmental factors. Multiple, complex molecular events characterize prostate cancer initiation, unregulated growth, invasion, and metastasis (Fig 2). Distinct sets of genes and proteins dictate progression from precursor lesion to localized disease and finally to metastatic disease. Clinically localized prostate cancer can be effectively ablated by using surgical or radiation treatments. Metastatic disease, however, is invariably incurable and leads to death. Androgen ablation is the most common therapy for advanced prostate cancer, leading to massive apoptosis of androgen-dependent malignant cells and temporary tumor regression. In most cases, however, the tumor re-emerges and can proliferate independent of androgen signals. With the advent of global profiling strategies, a systematic analysis of genes involved in prostate cancer is now possible. There are multiple key signaling pathways associated with prostate cancer progression. The androgen receptor (AR) plays a central role in any model, but other key pathways include PTEN, NKX3.1, MYC, and GST-pi (Fig 2).

Taken together, these molecular alterations represent events that may mutually add to the development and progression of prostate cancer. Although some investigators have favored a molecular model that includes linear accumulation of molecular lesions leading to prostate cancer progression,[6] we favor a working model that includes multiple nodes for progression (Fig 3). This view is supported by the clinical and molecular heterogeneity identified in prostate cancer. Work by LaPointe et al[7,8] and observations regarding gene fusion prostate cancer suggest that molecular alterations in prostate cancer do not accumulate in a linear manner but may, in fact, indicate differences in the ability to progress. As depicted in Figure 3, some molecular lesions may be seen in indolent tumors, whereas other tumors harboring a different set of alterations may progress to a metastatic state. Importantly, some molecular lesions may be associated with tumors that have little ability to progress beyond the in situ state. These theoretical considerations require the careful classification of tumors to aid in the determination of key factors in disease progression.

UNDERSTANDING PROSTATE CANCER HETEROGENEITY THROUGH GENE FUSIONS

The clinical and molecular heterogeneity of prostate cancer represents a major challenge in developing adequate diagnostic and prognostic tools and creates a major hurdle in drug development. We propose that recognition of the complexity of gene fusion prostate cancers will lead to a better classification of a disease that, until now, has been treated as a single entity.

Multiple Types of Gene Fusions in Prostate Cancer

Since the initial discovery of *ETS* fusions in prostate cancer, several recent studies have identified fusion events involving additional *ETS* family members (ie, *ELK4*,[9,10]) novel 5′ (ie, upstream)

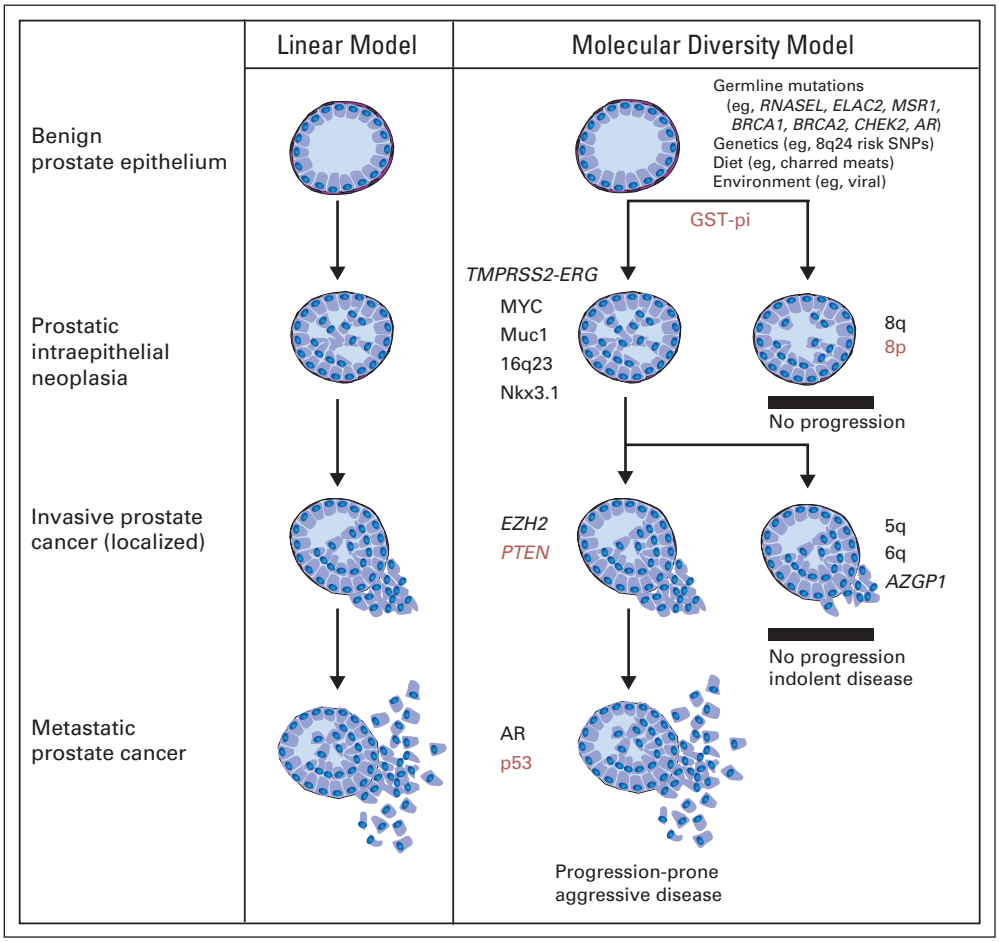

Fig 2. Two models of prostate cancer progression. The standard view has been that prostate cancer progresses through a series of molecular lesions. In the linear model, molecular events, including mutations, deletions, and amplifications, occur in sequence corresponding to progression of disease from the morphologically appearing benign prostate tissue, moving to high-grade prostatic intraepithelial neoplasia (PIN), then progressing to invasive prostate cancer, and finally to local and distant metastatic spread. However, in this review, we support the view that prostate cancer progresses through a wide range of lesions that lead to several possible pathways, some of which may not progress at all. In the molecular diversity model, alterations occur that might be classified as gatekeeper lesions. Once these events occur, additional events may lead to PIN that does not have the capacity to progress or PIN that may progress. Accumulation of molecular alterations associated with aggressive disease such as the overexpression of *EZH2* or *PTEN* mutations may lead to invasive disease that progresses to metastatic disease, whereas other lesions such as 5q or 6q gain and overexpression of *AZGP1* might be seen most often in indolent disease. Mutations and alterations associated with p53 and the androgen receptor (AR) are probably late events and may play a key role in the development of castration-resistant disease. SNP, single-nucleotide polymorphism.

partners, and a class of non–*ETS*-based fusions. On the basis of these discoveries, we have developed a classification system (Fig 4) comprising three categories: (1) fusions involving *ETS* gene family members (*ERG, ETV1, ETV4, ETV5,* and *ELK4*), (2) *RAF* kinase family fusions, and (3) *SPINK1*-positive cases.

The largest category, *ETS* fusions, is composed of the highly recurrent *TMPRSS2-ERG* fusion, which contrasts with the remaining less common fusion events. Interestingly, the *ETS* family member fusions involve a diverse set of 5′ upstream partners, as exemplified by *ETV1* having nine different fusion partners. In addition to *TMPRSS2*, three additional androgen responsive 5′ partners—*SLC45A3*,[11,12] *HERPUD1*,[13] and *NDRG1*[14]—have been found to fuse with *ERG*. However, many of the 5′ partners appear to fuse to multiple *ETS* family members, such *SLC45A3* (*-ERG, -ELK4, -ETV1,* and *-ETV5*) and *TMPRSS2* (*-ERG, -ETV1, -ETV4,* and *-ETV5*), both of which are androgen responsive. Overall, the emerging trend is that most of these organ-specific promoters are driven initially by AR signaling. Thus, one hypothesis worthy of testing is that patients who harbor an androgen-induced gene fusion might be more responsive to hormonal treatment than those who harbor a constitutively active or androgen-repressed promoter.

Recent advances in next generation transcriptome sequencing facilitated the discovery of the second category—*RAF* kinase gene fusions *SLC45A3-BRAF*, *ESRP1-RAF1*, and *RAF1-ESRP1* in advanced prostate cancers.[15] Although rare, detected in approximately 1% to 2% of prostate cancers, *RAF* kinase fusions represent the first "driver" fusions in prostate cancers that do not involve an *ETS* family member. The third category, *SPINK1*-positive prostate cancers, is included in the classification since the outlier expression of *SPINK1* occurs in *ETS* rearrangement–negative prostate cancers and therefore defines a specific subclass of prostate cancers.[16] We presume that this is a first-generation classification and that future iterations will include other non-*ETS* gene fusions as well as driving molecular mutations as they are discovered.

A CALL FOR A MOLECULAR SUBCLASSIFICATION OF PROSTATE CANCER

Like hematologic and pediatric tumors, many neoplasms are defined by the genetic rearrangement they harbor as the defining oncogenic event; we believe the fusion of an androgen-driven promoter and an *ETS* family transcription factor should be a defining molecular event in prostate cancer. Here, we present supporting evidence based on the key role of *ETS* genes as oncogenic, phenotypic changes associated with the *TMPRSS2-ERG* fusion, in vitro and in vivo cell data, the early nature of this molecular event, the association with an aggressive natural history in the absence of treatment, and the presence of a defined molecular signature to justify the classification of *TMPRSS2-ERG* fusion cancers as a distinct subclass. We hope that future clinical

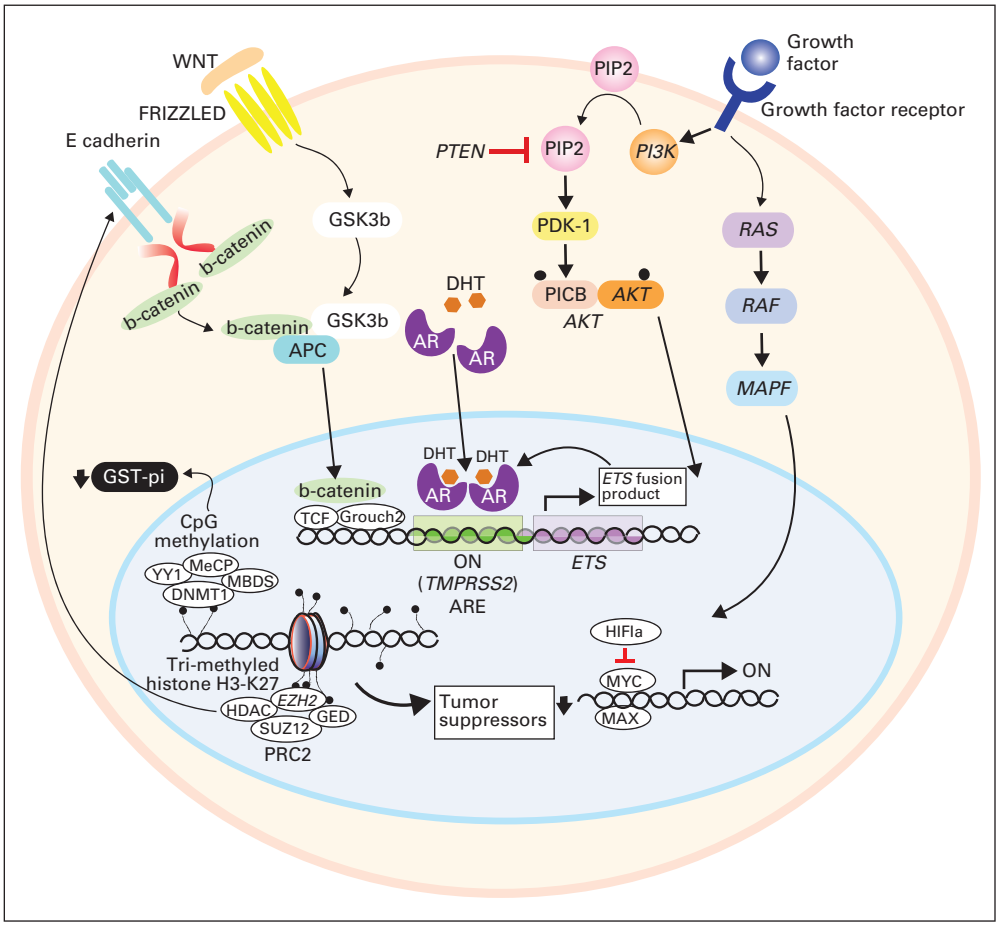

Fig 3. Molecular events associated with prostate cancer development and progression. Recent work has identified several genes and pathways associated with prostate cancer progression. Critical pathways depicted in this schematic of a prostate cancer cell include disruption of the WNT signaling, *PI3K/AKT/PTEN* and *RAS/RAF/MAPF* kinase pathways. Other pathways may be involved in the inactivation of GST-pi through methylation and histone methylation by polycomb genes such as *EZH2*. The activation of alterations of the androgen receptor (AR) is also believed to play a central role in disease progression from the androgen-dependent state to the castration-resistant state observed in advanced disease. The *ETS* fusion cancers often harbor an upstream, hormonally regulated promoter (eg, *TMPRSS2* or *SLC45A3*). These promoters are known androgen response elements (AREs) and act as amplifiers of the *ETS* gene expression in the setting of androgens. Interestingly, recent work has also demonstrated the presence of estrogen binding sites on the *TMPRSS2* promoter site (not depicted), suggesting a mechanism for continued expression of the *ETS* fusion transcript in the castration state of low androgens. DHT, dehydrotestosterone.

and molecular studies will take into account the *TMPRSS2-ERG* fusion status and other subtypes as they become more clearly defined.

TMPRSS2-ETS fusions occur early and are present in the precursor lesion high-grade prostatic intraepithelial neoplasia. Microscopic examination of prostate cancers by using a fluorescent in situ hybridization (FISH) assay reveal that gene fusion occurs in neoplastic cells but not in adjacent benign nuclei or stromal cells.[2,17,18] A larger study drawn from a wide spectrum of benign prostatic lesions and precursors of prostate cancer[19] failed to detect *TMPRSS2-ERG* fusion in benign prostate tissue, benign prostatic hyperplasia, or proliferative inflammatory atrophy (also commonly referred to as focal prostate atrophy or prostate atrophy; reviewed in De Marzo et al[20]). The *TMPRSS2-ERG* fusion was observed in approximately 20% of high-grade prostatic intraepithelial neoplasia (PIN) lesions intermingled with prostate cancer that carried the same fusion pattern. This was the same frequency previously detected by Cerveira et al[21] by using a reverse transcriptase polymerase chain reaction (RT-PCR) –based assay. We did not observe the *TMPRSS2-ERG* fusion in high-grade PIN lesions geographically distant to prostate cancer, even if the prostate cancer from the same individual demonstrated the *TMPRSS2-ERG* fusion. More recently, immunohistochemistry has been used to evaluate the gene fusions in situ.[22] By using an antibody highly specific for *ERG* rearrangements, one can clearly see the earliest overexpression of the *ERG* oncogene in the morphologic area of high-grade PIN but not in directly adjacent benign prostate tissue (Fig 5). Hence, we believe these high-grade PIN lesions are a subset of true precursors for *TMPRSS2-ERG*–positive prostate cancer. A significant clinical implication for this finding is the potential utility of assessing the *TMPRSS2-ERG* fusion status in problematic prostate needle core biopsies with high-grade PIN and adjacent small atypical glands.

Prevalence of Gene Fusions in Prostate Cancer

Several independent studies[8,21,23-35] have corroborated the initial observation that *TMPRSS2-ETS* fusions are common in prostate cancer. Although most studies have focused on the dominant rearrangement *TMPRSS2-ERG* fusion, a variety of other fusions involving *TMPRSS2* and other 5′ partners have been described (Fig 4) but appear to be less common.[5,33,36-38] The prevalence of *TMPRSS2-ERG* prostate cancer has been reported to range from 40% to 70%, depending on the clinical cohorts investigated. The first large clinical study on a German prostatectomy cohort[17] reported that approximately 50% of cases had a *TMPRSS2-ERG* fusion. Several retrospective studies[24,30,32,36,39,40] from PSA-screened prostatectomy cohorts have reported frequencies of the *TMPRSS2-ERG* fusion between 35% and 50% when FISH assays were used to detect the rearrangement. Other smaller studies[8,21,23,28,29] that used PCR-based methodology have reported higher frequencies. Only one study to date[36] has comprehensively explored for the presence of other fusion partners and determined that an additional 5% to 10% of cases may harbor other gene fusions, including *TMPRSS2-ETV1* and *TMPRSS2-ETV4*.

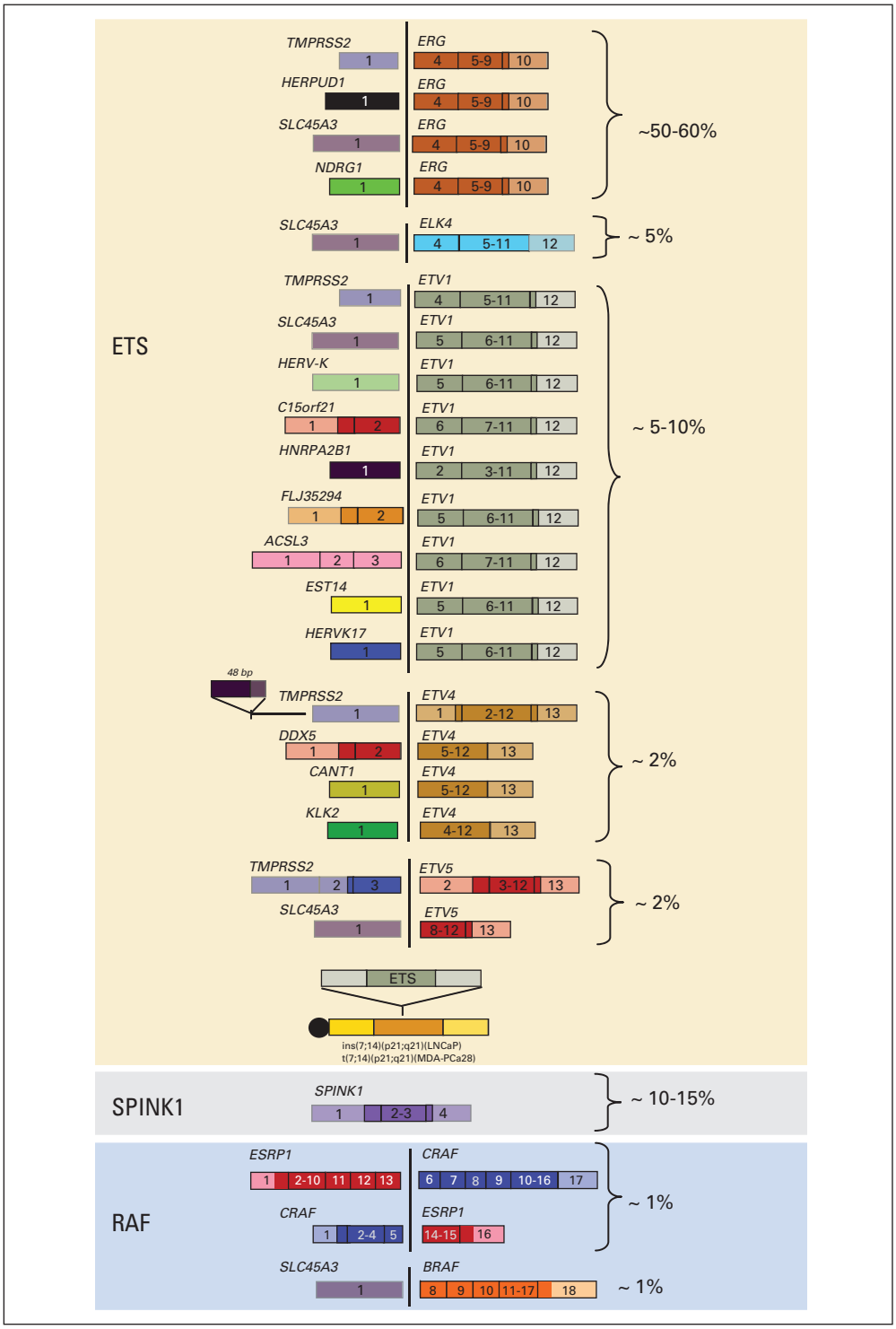

Fig 4. Prostate cancer gene fusion classification. The ongoing effort to screen prostate cancer patients for gene fusions, in combination with the recent technology advances, has resulted in a comprehensive gene fusion landscape. This schematic highlights all published gene fusions categorized into ETS rearrangements, *RAF* kinase gene fusions, and SPINK1-positive, ETS rearrangement–negative prostate cancers. The percentages highlight the estimated frequency of each gene fusion on the basis of published screens.

In two population-based studies from Sweden and the United Kingdom,[30,41,42] 15% to 20% of men diagnosed with incidental prostate cancer had tumors that harbored *TMPRSS2-ERG*. It is worth highlighting that the 354 incidental cancers from the Swedish cohorts were detected in five population-based cohorts before PSA screening.[41] All of the tumors were detected on transurethral resection of the prostate (TURP) samples, which differs from the prostatectomy series. Although some have suggested that there may be a genetic component to this lower frequency in the Swedish population, we have determined that the frequency in a PSA-screened biopsy cohort from Örebro is approximately 45%, which is similar to that in all other PSA-screened hospital-based cohorts (Svensson and Rubin, manuscript in preparation). We have observed similar frequencies in gene fusion when examining hormone-naive primary and castration-resistant metastatic prostate cancers.[43]

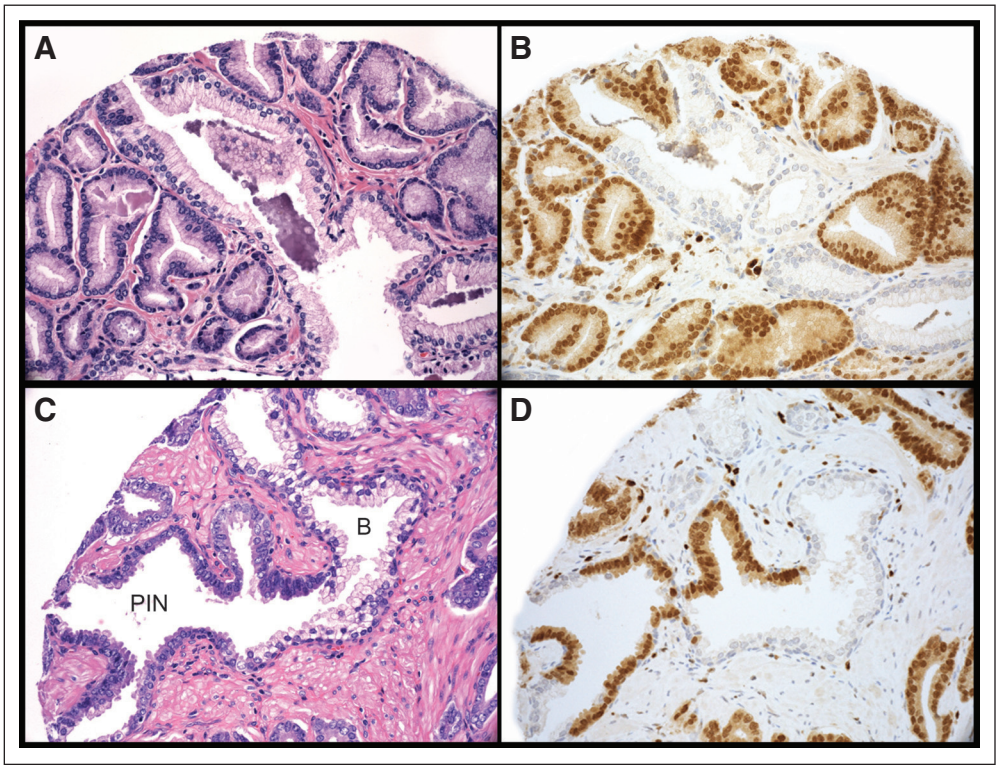

Fig 5. *ERG* rearranged prostate cancer and high-grade prostatic intraepithelial neoplasia (HGPIN) express high levels of truncated ERG protein. Representative examples of prostate cancers and HGPIN (A and C) showing similar ERG protein expression by immunohistochemistry (a rabbit anti-ERG monoclonal antibody, clone EPR 3864, Epitomics, Burlingame, CA). Hematoxylin and eosin stain demonstrates prostatic cancerous glands (A) and another case with HGPIN (C). ERG protein expression by immunohistochemistry demonstrates strong expression in both cancer (B) and HGPIN (D). In (C), the PIN label indicates discrete demarcation between HGPIN and histologically benign luminal epithelial cells labeled B (×40).

As part of an Early Detection Research Network (EDRN) study sponsored by the National Cancer Institute, we prospectively determined that 46% of men with prostate cancer detected on 12 core needle biopsies by PSA screening harbor *TMPRSS2-ERG* fusion.[44] This result is consistent with results in the surgical cohorts. Taken together, observations made over the past 3 years from several studies since the original description of *TMPRRSS2-ETS* prostate cancer suggest that the majority of prostate cancers currently detected by PSA screening harbor either the common *TMPRSS2-ERG* fusion (46%) or one of the less common fusions involving *TMPRSS2* or other 5′ partners (5% to 10%). This has important clinical implications, because the *TMPRSS2-ERG* transcript can be detected in urine and represents a highly specific prostate cancer biomarker.

TMPRSS2-ERG *and Association With a More Aggressive Clinical Outcome*

The data generated in the search for associations with clinical outcome emerge from two types of studies: population-based watchful waiting cohorts and retrospective prostatectomy series. A review of the literature suggests that, in some instances, the *TMPRSS2-ERG* fusion is associated with a more aggressive clinical course but, conversely, others report the opposite result. We hope to clarify this confusion but concede that large population-based studies will be required to clarify this issue in the future.

Our group initially observed an enrichment in the *TMPRSS2-ERG* fusion in higher-stage prostate cancer.[17] We then searched for associations between *TMPRSS2-ERG* fusion and clinical outcome in a population-based study.[42] The Örebro watchful waiting cohort represents a treatment-naive population drawn from a strictly defined catchment area for 190,000 inhabitants living in Örebro. The *TMPRSS2-ERG* gene fusion was identified in 15% (17 of 111) of the patients' initial TURP biopsy samples and was significantly associated with prostate cancer–specific death (cumulative incidence ratio, 2.7; 95% CI, 1.3 to 5.8; $P < .01$). This is a well-defined population that dramatically differs from that in the retrospective prostatectomy series. First, this is a population-based cohort. All men with early prostate cancer (T1a-b, Nx, M0) diagnosed by TURP for symptomatic benign prostatic hyperplasia (ie, lower urinary tract symptoms) were included. There was no PSA screening in Sweden during the collection phase of this study. Second, the patients were followed expectantly (without curative treatment) and received clinical examinations, laboratory tests, and bone scans every 6 months during the first 2 years following diagnosis and subsequently at 12-month intervals. Third, the end point of the study was lethal prostate cancer, defined as development of distant metastases or prostate cancer as the underlying cause of death (median follow-up time, 9.1 years; maximum, 28 years). Therefore, this unique study design allows one to assess the biologic impact of *TMPRSS2-ERG* prostate cancer in the absence of early intervention.

The results of this study were supported by a report from the United Kingdom[30] that identified associations between *TMPRSS2-ERG* fusion and survival of 445 men conservatively treated for prostate cancer. Overall, cancers lacking *TMPRSS2-ERG* fusion alterations demonstrated 90% survival at 8 years of clinical follow-up. The report also identified a novel association seen in *TMPRSS2-ERG* fusion prostate cancer in which the fusion of *TMPRSS2* to *ERG,* along with interstitial deletion of sequences 5′ to *ERG,*[17] was associated with a significantly worse cause-specific survival that took into account age, Gleason score, and pretreatment PSA. Supporting the hypothesis that overexpression of *ERG* is acting as an oncogene, the overall lowest cause-specific survival was associated with a duplication of the *TMPRSS2-ERG* fusion with an accompanying interstitial deletion

(hazard ratio, 6.10; 95% CI, 3.33 to 11.15; $P < .001$; 25% survival at 8 years). On multivariate analysis, the duplication of the *TMPRSS2-ERG* fusion with associated deletion (referred to as "2+Edel") was an independent predictor of clinical outcome that provided information in addition to Gleason score and pretreatment PSA level.

This study reported on 110 clinical T1 prostate cancer cases that had 20% *TMPRSS2-ERG* fusion similar to that in the Swedish watchful waiting cohort. This study supports the aggressive biologic significance of the *TMPRSS2-ERG* fusion. Two key observations from this study were that gain of *ERG* and the associated interstitial deletion of the 3-Mb region between *TMPRSS2* and *ERG* on chromosome 21 are associated with more aggressive prostate cancer. Overexpression of *ERG* has been associated with poor clinical outcome in acute myeloid leukemia,[45] and some of the genes located in the 3-Mb area of deletion (eg, *HMGN1, ETS-2*) may be acting as tumor suppressor genes.[17]

Several retrospective studies[29,31,35] that sought an association between *TMPRSS2-ERG* and outcome following radical prostatectomy gave mixed results. It is difficult to compare results from a surgical study that used PSA biochemical failure as an end point with one that used observational studies with cancer-specific death as an outcome. One of the limitations of using an increase in PSA following prostatectomy as a surrogate end point comes from a single-institution study of men diagnosed with clinically localized prostate cancer in the pre-PSA-screening era. Porter et al[46] observed 45.5% PSA biochemical failure in a radical prostatectomy series, but prostate cancer–specific death occurred in only 18.5% of the population with a follow-up time of up to 25 years. Carver et al[47] reported that, in a population of high-risk men with T3 prostate cancer who underwent radical prostatectomy, 36% with PSA biochemical failure subsequently developed clinically relevant disease progression. Ward et al[48] found that in a population of 3,897 radical prostatectomy patients, only 8.3% of the men with PSA biochemical failure died of prostate cancer with a median follow-up time of 10 years. An increase in PSA following surgery is associated with prostate cancer–specific death, but the majority of men with PSA biochemical failure will die of other causes. Therefore, we would argue for caution in overinterpreting the results of each of these types of clinical studies.

On the basis of the two large observational clinical studies with long-term follow-up, we would argue that left untreated, *TMPRSS2-ERG* prostate cancer will run a more aggressive clinical course than fusion-negative cancer. In the setting of surgical or other interventions immediately following diagnosis, there is insufficient data to make any reasonable conclusions.

Gene fusion is a key molecular event in prostate cancer development. Initial work exploring the role of the *TMPRSS2-ERG* fusions in cell lines demonstrates fairly consistent findings for overexpression of the *ETS* gene in benign epithelial cells. Studies that have overexpressed *ETV1, ETV5,* and *ERG* have demonstrated an increase in cell invasion capability, not an increase in proliferation or the ability to transform these cells into tumor cells.[37,38,49] This was recently confirmed by Klezovitch et al,[50] who demonstrated that the overexpression of *ERG* is associated with tumor cell migration through a proteolytic molecular program. These results suggest that *ETS* genes alone are insufficient to cause a transformation to cancer but may play a key role in the development of the invasive phenotype in the context of other underlying molecular alterations. It is also possible that these models do not capture the complexity of deregulation due to the fusion events. For example, could the decreased expression of *ETS-2,* located in the minimally deleted region of a translocated allele, in conjunction with *ERG* overexpression play a different role in vivo?

There are several published and unpublished mouse models that have been generated to recapitulate the overexpression of *ERG*[49,50] and *ETV1.*[37] All of these models demonstrate the ability of the trans gene to develop early molecular changes referred to as mouse PIN.[51] These subtle changes have not reached the level of invasive cancer.[52] This is similar to models of NKX3.1 and PTEN. Therefore, more recent efforts have focused on the identification of cooperating events in *ETS*-induced prostate carcinoma to rationalize combined therapies. For instance, Zong et al[52] demonstrated that *ERG* overexpression cooperates with PI3K signaling to progress to invasive prostate adenocarcinoma. In addition, the combination of overexpressing both AR and *ERG* promoted the development of poorly differentiated invasive adenocarcinomas. These promising results support ongoing work to further elucidate the combination of other known prostate cancer oncogenes and to explore a cumulative effect. Therefore, the in vitro and in vivo models demonstrate that *ETS* genes have an effect on tumor progression but alone do not appear to be sufficient for transformation into cancer.

Gene fusion is a clonal event that aids understanding of prostate cancer heterogeneity. It is recognized that prostate cancer is multifocal. Both morphologic and molecular analysis have shown that by the time prostate cancer is diagnosed, more than 80% of prostates harbor multiple separate cancer foci.[53-57] These discrete lesions have both biologic and clinical implications. The *TMPRSS2-ERG* fusion represents an excellent early clonal marker to provide insight into molecular heterogeneity.

TMPRSS2-ERG fusions, when present, are distributed evenly among all tumor nuclei within a discrete tumor lesion. We reported that 243 of 246 prostate cancer cases demonstrated homogeneity within a discrete tumor nodule.[17] This observation was extended when multiple microdissected foci of cancer from individual patients were examined by RT-PCR for gene fusions and demonstrated that either all or no foci overexpressed *ERG* and its family members *ETV1* and *ETV4.*[58] Thus, within a discrete nodule, the fusion rearrangement must occur early because all of the tumor nuclei harbor the fusion when present. However, when we undertook studies to evaluate rearrangement among the multiple nodules within a single prostate gland from one individual, we found that discrete lesions may occur independently from one another. This has been observed in three independently conducted studies.[59-61] For example, in the study by Barry et al,[60] 32 prostatectomy samples with clear-cut discrete tumors demonstrated fusion by balanced translocation and fusion by interstitial deletion occurring as distinct events, suggesting that these are clonal mechanisms for achieving *TMPRSS2-ERG* fusion. Interestingly, that study found a high rate of interfocal heterogeneity for fusion status (41%). These observations have both biologic and clinical implications. Biologically, the presence of multiple clonally distinct lesions suggests that, within a single gland, complex molecular events such as gene rearrangement can occur in some but not all lesions. This makes classifying prostate cancers more challenging. From a clinical perspective, how does one determine the most aggressive nodule to target? It has long been assumed that the dominant nodule harbors the most aggressive tumor and therefore dictates the clinical course. Therefore, if *TMPRSS2-ERG* prostate cancers are more biologically aggressive, strategies will be needed to detect them regardless of their size because these may be the tumors with the highest propensity for metastatic dissemination.[44]

DIAGNOSTIC AND CLINICAL THERAPY IMPLICATIONS

PSA has a diminished role in detecting prostate cancer, thus the requirement for a new molecular detection test. Several studies[62-64] to date have demonstrated the detection of the *TMPRSS2-ERG* fusion transcripts in urine. These studies and other unpublished reports demonstrate a high specificity. Unlike PSA, which can be increased in benign conditions as well as in cancer, the presence of *TMPRSS2-ERG* transcripts has been reported only in neoplastic cells. In addition to the sensitive and specific detection of *TMPRSS2-ERG* in urine sediment,[64] recent work has demonstrated improved detection of prostate cancer by using multiple biomarkers. Multiplexed detection of *GOLM1*, *SPINK1*, *PCA3*, and *TMPRSS2-ERG* was a more significant predictor of prostate cancer than serum PSA or PCA3 alone.[64] These results are promising and, with some refinement, could be adopted as a clinical supplement to serum PSA for prostate cancer detection.

Given the heterogeneity demonstrated between tumor nodules, a positive *TMPRSS2-ERG* urine test and a biopsy negative for cancer would suggest that the cancer has been missed. If the cancer is detected but is fusion negative, the sampling would have missed the fusion cancer. The finding of interfocal heterogeneity for *TMPRSS2-ERG* fusion has direct relevance in the context of a urine test result that is positive for fusion and a subsequent prostate biopsy with cancer that is negative for fusion. Given the potential prognostic role of determining the mode of rearrangement (deletion through translocation *v* through interstitial deletion), a biopsy FISH test would allow for an accurate determination of the presence and type of gene fusions (Fig 6).

Recent trials in the setting of castration-resistant prostate cancer suggest that targeting androgen and estrogen might be an effective approach. Data suggest that low levels of intraprostatic testosterone or dehydrotestosterone are still present when men have undergone chemical castration with antiandrogens. Therefore, novel approaches have been developed to reduce these low levels of androgens and estrogens by blocking steroid synthesis. Abiraterone acetate is a selective small-molecule inhibitor of cytochrome P450 17 (CYP17), which effectively blocks the production of androgen and estrogen.[65] It was recently tested in a phase I clinical trial, and it demonstrated a decrease in PSA following treatment in 50% of all men with castration-independent prostate cancer.[66,67] In that study, 83% of men (5 of 6) with *TMPRSS2-ERG* fusion prostate cancer had a decrease in PSA following abiraterone treatment. Although that study was not designed to test the potential role of abiraterone with respect to *TMPRSS2-ERG* fusion status, future phase II and III studies will examine this hypothesis on the basis of these initial observations.

The *RAF* kinase fusions, although rare, are of immediate therapeutic significance given the numerous approved and investigational agents in the late stage of development. Palanisamy et al[15] demonstrated that the *RAF* kinase fusions were sensitive to sorafenib, a US Food and Drug Administration (FDA) –approved *RAF* inhibitor that has also been demonstrated to target additional kinases.[68] This suggests that screening patients for *RAF* fusions may identify a subset of the population that may benefit from existing targeted therapies similar to the current clinical application of *ALK* inhibitors to patients with *EML-ALK4* non–small-cell lung carcinoma.[69,70] We envision that other targetable gene fusions and driving mutations will be discovered in the coming years.

Ateeq et al[71] recently demonstrated that *SPINK1* prostate cancer can be targeted by using cetuximab, an epidermal growth factor receptor (EGFR) inhibitor. *SPINK1* harbors a high homology with EGF. Preclinical models that use recombinant *SPINK1* support targeting the extracellular domain of *SPINK1*. This early work provides a rationale for both the development of humanized monoclonal antibodies to *SPINK1* and evaluation of EGFR inhibition in *SPINK1*-positive/*ETS*-negative prostate cancers.

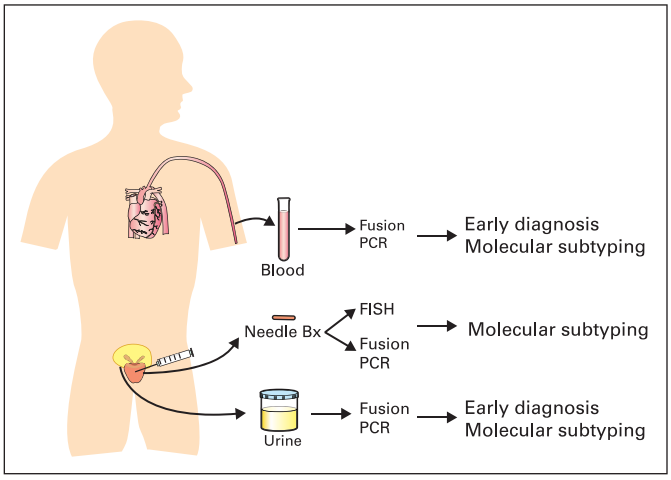

Fig 6. The diagnostic predictive and prognostic implication of *ETS* fusion prostate cancer. The fusion of two genes to form a novel chimeric mRNA transcript represents a unique opportunity to develop a diagnostic test. Recent studies have demonstrated that the fusion transcript can be identified in the serum and urine from men with prostate cancer. The urine assay is being developed commercially with the goal of establishing a highly specific test. Prostate tissue derived from clinical biopsies, transurethral resection of the prostate samples, or radical prostatectomies can be used to detect the *ETS* rearrangement events by using fluorescent in situ hybridization (FISH) or reverse transcriptase polymerase chain reaction (PCR). The identification of *ETS* rearrangements may have prognostic implications in specific settings (eg, an active surveillance clinical trial) and may also be predictive of response to targeted therapies such as those targeting the androgen receptors. The significance of these clinical assays will largely depend on future studies that determine to what extent *ETS* rearrangement prostate cancers behave differently from nonrearranged prostate cancers. Bx, biopsy.

EMERGING UNDERSTANDING OF PROSTATE CANCER GENOMIC COMPLEXITY

The emerging picture of prostate cancer genomic complexity demonstrates numerous rearrangements including the well-described *ETS* rearrangements.[72] Some of these complex genomic alterations might lead to deregulation of important signaling pathways such as the *MAGI2* inversions described by Berger et al[72] that putatively lead to *AKT* activation. Understanding the underlying cause of these rearrangements may play a role in chemoprevention or selection of chemotherapies.

Genomic rearrangements appear to be nonrandom and locus-specific, and they depend, in part, on the proximity of chromosomal regions in the nucleus.[73] Moreover, there is mounting evidence suggesting that transcription factors are associated with DNA double-strand breaks, thus predisposing transcribed regions to genomic rearrangements. For example, both androgen and estrogen signaling recruit the enzyme topoisomerase-2 beta (TOP-2b) to target gene promoters, which creates DNA double-strand breaks and facilitates transcription.[74,75] AR and TOP-2b are coexpressed in human prostate cancer precursor lesions in which *TMPRSS2-ERG* rearrangements

are known to occur, suggesting a critical role of TOP-2b in the recurrent *ETS* rearrangements. Three recent studies[76-78] have also shown that androgen signaling promotes *TMPRSS2-ERG* fusion formation, in part, by recruiting DNA break-inducing enzymes such as activation of induced cytidine deaminase to translocation breakpoint sites.[77] More recently, we demonstrated that rearrangement breakpoints were enriched near open chromatin, AR, and *tERG* DNA binding sites in the setting of the *ETS* gene fusion *TMPRSS2-ERG* but were inversely correlated with these regions in tumors lacking *ETS* fusions.[72] Hence, transcription factors can contribute to the formation of genomic rearrangements by facilitating the juxtaposition of chromosomal loci and recruiting enzymatic machinery involved in DNA breaks to these target loci. This work also suggests that inhibitors of repair enzymes such as PARP1 and DNA-PK decrease the susceptibility to gene fusions. It also raises concerns that TOP-2b inhibitors such as etoposide or doxorubicin might facilitate gene fusions and rearrangements by enhancing double-stranded DNA breaks. Ongoing research is exploring the clinical implications of these observations.

In conclusion, gene fusion prostate cancer is among the most common genetic alterations identified in cancer. Although several *ETS* and non-*ETS* family members have been observed to be fused with *TMPRSS2* or other 5′ partners, the vast majority of fusions involve *TMPRSS2-ERG*. This fusion can easily be studied, because it was identified in approximately 50% of all prostate cancers screened for PSA. Associations with disease-specific death have been made in clinical observation studies. The amplification of the *TMPRSS2-ERG* fusion and the interstitial deletion associated with the translocation add additional statistical power to predicting lethal prostate cancer. Morphologic features, functional in vitro and in vivo studies, and a specific gene signature support the view that the *TMPRSS2-ERG* fusion cancers represent a distinct molecular subclass. The more recent discovery of the *RAF* fusions also demonstrates that some of the gene fusions will be targets for clinical intervention.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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